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Antigenic and Genetic Characterization of Influenza C Viruses Which Caused Two Outbreaks in Yamagata City, Japan, in 1996 and 1998

Y. Matsuzaki,[1]* K. Sugawara,[1] K. Mizuta,[2†] E. Tsuchiya,[1] Y. Muraki,[1] S. Hongo,[1] H. Suzuki,[3] and K. Nakamura[1]

Department of Bacteriology, Yamagata University School of Medicine, Iida-Nishi, Yamagata 990-9585,[1] Virus Research Center, Clinical Research Division, Sendai National Hospital, Sendai 983-8520,[2] and Department of Public Health, Niigata University School of Medicine, Asahi-Machi Dori, Niigata 951-8510,[3] Japan

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During the 3 years from January 1996 to December 1998, a total of 33 strains of influenza C virus were isolated from 10,726 throat swab specimens collected from children with acute respiratory illness who visited two pediatric clinics in Yamagata City, Japan. These 33 strains were isolated in clusters during two different periods, 20 strains in May to August 1996 and the remaining 13 in March to June 1998. Antigenic analysis with monoclonal antibodies to the hemagglutinin-esterase (HE) glycoprotein and phylogenetic analysis of seven RNA segments showed that the 33 influenza C viruses isolated were antigenically and genetically similar and that they were reassortant viruses which had obtained PB2, PB1, HE, M, and NS genes from a C/pig/Beijing/115/81-like virus and P3 and NP genes from a C/Mississippi/80-like virus. These observations suggest strongly that during the survey period of 3 years, two outbreaks of influenza C occurred in Yamagata City, both of which were caused by a reassortant virus having the genome composition described above.

Influenza C virus usually causes a mild upper respiratory illness (11) but can also cause lower respiratory infections, such as bronchitis and pneumonia (19). Although seroepidemiological studies revealed that influenza C virus is widely distributed throughout the world (5, 9, 10, 22), outbreaks of illness caused by the virus have rarely been detected, and the virus has been isolated only occasionally (6, 7, 8, 11, 12, 18). Therefore, information about the epidemiology of influenza C is scarce compared to that for influenza A and influenza B.

The genome of the influenza C virus consists of seven RNA segments, which encode three polymerase proteins (PB2, PB1, and P3), hemagglutinin-esterase (HE) glycoprotein, nucleoprotein (NP), matrix (M1) protein, and two nonstructural proteins (NS1 and NS2) (reviewed in reference (14). Thus, reassortment characterized by exchange of genome segments between two different influenza C virus strains occurs not only in vitro (24) but also in nature (2, 17, 27, 32). However, the epidemiological significance of genetic reassortment in influenza C viruses is unknown.

To obtain more information about influenza C epidemiology, we developed a tissue culture method for primary virus isolation that is convenient for routine work with a large number of specimens (19, 23) and then initiated surveillance for influenza C virus infections in Yamagata City in 1988 and in the adjacent city of Sendai in 1990. We compared the antigenic specificity of the HE glycoprotein as well as the HE gene sequence among 42 strains isolated between 1947 and 1993 (which included 13 strains obtained by our surveillance work) and revealed the existence of six distinct virus groups, represented by strains Taylor/47, Kanagawa/1/76 (KA176), Yamagata/26/81 (YA2681), Aichi/1/81 (AI181), Sao Paulo/378/82 (SP82), and Mississippi/80 (MS80), four of which (YA2681-, AI181-, SP82-, and MS80-related lineages) circulated in Japan in the 1980s and the early 1990s (17).

We report here the results of a 3-year survey (January 1996 to December 1998) performed in Yamagata City. The data show that during the 3 years, two outbreaks of influenza C occurred in the city, in May to August 1996 and March to June 1998, both of which were caused by reassortant viruses having the same genome composition that emerged from two parental viruses closely related to strains pig/Beijing/115/81 (a virus having the HE gene from the YA2681-related lineage) and MS80.

MATERIALS AND METHODS

Viruses and cells. A total of 33 strains of influenza C virus (listed in Table 1) isolated from pediatric patients (<15 years of age) with acute respiratory illness who visited Katsushima Pediatric Clinic (located in Yamagata City) or Yamagata City Hospital Saiseikan during a 3-year period from January 1996 to December 1998 were used. All these viruses were initially isolated from throat swab specimens using the HMV-II line of human malignant melanoma cells or Madin-Darby canine kidney (MDCK) cells as a host according to described procedures (19, 23). For this study, however, viruses were reisolated from throat swabs by inoculating them into the amniotic cavity of 9-day-old embryonated hen's eggs, because isolation and passages of influenza C virus in tissue culture cells sometimes result in selection of antigenic variants (33).

Six older strains (KA176, Miyagi/77 [MI77], MS80, YA2681, AI181, and SP82) as well as 12 strains (Miyagi/1/93 [MI193], Miyagi/2/93 [MI293], Miyagi/3/93 [MI393], Miyagi/4/93 [MI493], Miyagi/6/93 [MI693], Miyagi/7/93 [MI793], Miyagi/1/94 [MI194], Miyagi/2/94 [MI294], Miyagi/3/94 [MI394], Miyagi/4/96 [MI496], Miyagi/7/96 [MI796], and Miyagi/8/96 [MI896]) which had been isolated in Sendai City between 1993 and 1996 and found to possess HE antigenicity identical to that of strain MS80 were also used for comparison. These viruses were also isolated, passaged, and propagated in eggs. HMV-II cells were grown in RPMI 1640 medium containing 10% fetal calf serum, and MDCK cells were cultured in Eagle's minimal essential medium supplemented with 10% fetal calf serum.

* Corresponding author. Mailing address: Department of Bacteriology, Yamagata University School of Medicine, Iida-Nishi, Yamagata 990-9585, Japan. Phone: 81-23-628-5249. Fax: 81-23-628-5250. E-mail: matuzaki@med.id.yamagata-u.ac.jp.

† Present address: Yamagata Prefectural Institute of Public Health, Toka-Machi, Yamagata 990-0031, Japan.

TABLE 1. Antigenic analysis of influenza C virus strains isolated in Yamagata City in 1996 and 1998

Virus strain	Date of sample collection (mo. day)	HI titer[a] with MAb:			
		J14	Q5	U4	MS22
Reference strains					
Yamagata/26/81		32,000	3,200	20	<
Aichi/1/81		256,000	40	1,280	<
Mississippi/80		102,400	<	<	6,400
Sao Paulo/378/82		64,000	32,000	3,200	25,600
1996 isolates					
Yamagata/1/96	5.23	32,000	12,800	10	<
Yamagata/2/96	5.30	8,000	3,200	20	<
Yamagata/3/96	6.3	16,000	6,400	10	<
Yamagata/4/96	6.5	4,000	3,200	<	<
Yamagata/5/96	6.5	8,000	1,600	<	<
Yamagata/16/96	6.10	64,000	12,800	10	<
Yamagata/6/96	6.10	64,000	12,800	20	<
Yamagata/17/96	6.12	32,000	12,800	10	<
Yamagata/7/96	6.13	16,000	6,400	20	<
Yamagata/8/96	6.13	16,000	3,200	<	<
Yamagata/18/96	6.14	16,000	3,200	<	<
Yamagata/9/96	6.17	32,000	12,800	20	<
Yamagata/19/96	6.18	32,000	12,800	10	<
Yamagata/10/96	6.19	16,000	6,400	40	<
Yamagata/11/96	6.22	32,000	12,800	10	<
Yamagata/12/96	6.24	6,400	32,000	<	<
Yamagata/13/96	6.27	16,000	32,000	<	<
Yamagata/14/96	6.27	16,000	3,200	20	<
Yamagata/15/96	7.9	16,000	3,200	<	<
Yamagata/20/96	8.6	16,000	6,400	<	<
1998 isolates					
Yamagata/2/98	3.27	16,000	3,200	<	<
Yamagata/3/98	4.3	16,000	6,400	10	<
Yamagata/4/98	4.9	32,000	12,800	20	<
Yamagata/5/98	4.14	32,000	8,000	20	<
Yamagata/6/98	4.15	8,000	1,600	<	<
Yamagata/7/98	4.17	8,000	6,400	10	<
Yamagata/8/98	4.20	16,000	6,400	10	<
Yamagata/9/98	4.22	8,000	3,200	<	<
Yamagata/10/98	5.1	16,000	12,800	20	<
Yamagata/11/98	5.6	64,000	12,800	20	<
Yamagata/12/98	5.22	32,000	12,800	20	<
Yamagata/13/98	6.8	32,000	6,400	10	<
Yamagata/14/98	6.8	32,000	6,400	10	<

HI test. The hemagglutination inhibition (HI) test was done in microtiter plates with anti-HE monoclonal antibodies (MAbs) (30, 31) and 0.5% chicken erythrocytes. Briefly, 50 μl of 16 hemagglutinating units (HAU) of virus suspension per ml was added to each well containing 50 μl of twofold-diluted MAbs. After incubation for 30 min at room temperature, 100 μl of 0.5% chicken erythrocytes was added to all wells, and plates were stored for 60 min at 4°C. The HI titer was expressed as the reciprocal of the highest antibody dilution which completely inhibited hemagglutination. Precautions over inhibitors were not necessary because little or no inhibitor activity was detected in mouse ascitic fluid.

Nucleotide sequencing and phylogenetic analysis. Viral RNA was extracted from 200 μl of the virus-containing amniotic fluid with the RNeasy minikit (Qiagen). The viral RNA was then transcribed into cDNA with avian myeloblastosis virus reverse transcriptase XL (Life Science) and an oligonucleotide primer complementary to positions 1 to 12 at the 3′ end of all influenza C virus RNA segments. By using the resulting cDNA as a template, the individual RNA segments were amplified by PCR through 35 cycles of the thermocycler program described before (13). The PCR products were purified by rapid gel filtration with a Chroma Spin column (Clontech) and then sequenced by using the BigDye terminator cycle sequencing FS ready reaction kit on an ABI Prism 310 (Applied Biosystems) automatic sequencer.

The nucleotide sequences of the oligonuclotide primers used for PCR amplification and sequencing are available from the authors upon request. Sequence data were analyzed with the PHYLIP program (version 3.54c), and phylogenetic trees were constructed by the neighbor-joining method (29) using the same software.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been submitted to the DDBJ/EMBL/GenBank databases and assigned accession numbers AB064397 to AB064488.

RESULTS

Isolation of influenza C viruses. During a 3-year survey from January 1996 to December 1998, a total of 10,726 throat swab specimens were collected from children with acute respiratory illness and examined for the presence of influenza C virus. The results obtained are shown in Fig. 1. In 1996, 20 strains of influenza C virus were isolated between 23 May and 6 August. It was impressive that 16 of the 20 isolates were obtained during a short period of 24 days (from 3 to 27 June; see Table 1), the isolation rate of June 1996 being as high as 4.9% (16 of 328).

In 1997, influenza C virus was not isolated at all despite the examination of 3,550 throat swab specimens. In 1998, 13 strains were isolated between 27 March and 8 June, 7 of which were obtained in April, with an isolation rate of 2.8% (7 of 250). These observations suggest that during the 3 years (1996 to 1998), two outbreaks of influenza C may have occurred in Yamagata City, the first one in May to August 1996 and the second one in March to June 1998. Clinical details of the 33 children were largely similar to those described in our previous papers (11, 19). Briefly, fever, cough, and nasal discharge were observed in more than half of them, but lower respiratory tract infections such as bronchitis and pneumonia developed occasionally.

Antigenic analysis of influenza C viruses isolated in Yamagata City in 1996 and 1998. The 33 influenza C virus strains isolated in Yamagata City in 1996 and 1998 were examined in HI tests for reactivity with four different anti-HE MAbs characterized previously (30, 31). As shown in Table 1, the reactivity patterns of the 1996 and 1998 isolates were similar; all the viruses were highly reactive with MAbs J14 and Q5 but were unreactive or very weakly reactive with U4 and MS22. Comparison of this reactivity pattern with those of four reference strains (which represent each of the four virus groups shown to have circulated in Japan in the 1980s and the early 1990s [17]) revealed that all 33 isolates obtained from Yamagata City in 1996 and 1998 belonged to the YA2681 virus group.

Phylogenetic analyses of individual RNA segments of influenza C virus strains isolated in Yamagata City in 1996 and 1998. In order to confirm the results of the antigenic analysis, which suggested that all the 1996 and 1998 isolates from Yamagata City have HE genes belonging to the YA2681-related lineage, the sequence of the HE gene (nucleotides 64 to 1989) was determined for 7 (Yamagata/3/96 [YA396], Yamagata/8/96 [YA896], Yamagata/9/96 [YA996], Yamagata/20/96 [YA2096], Yamagata/2/98 [YA298], Yamagata/6/98 [YA698], and Yamagata/13/98 [YA1398]) of the 33 isolates, and a phylogenetic tree was constructed by using these 7 sequences in addition to the 42 published sequences (1, 3, 4, 13, 15, 16, 17, 20, 21, 25, 26, 27, 28, 33) as well as the 12 sequences of the 1993 to 1996 isolates from Sendai City determined here (see below for details).

The nucleotide sequences of the HE genes of the seven 1996 and 1998 isolates from Yamagata were strikingly similar, with sequence identities of 99.9% among the 1996 strains, 99.7 to 100% among the 1998 strains, and 99.7 to 100% between the 1996 and 1998 strains. As shown in Fig. 2, the HE genes of the influenza C viruses analyzed were divided into six discrete lineages, represented by Taylor/47, AI181, SP82, KA176, YA2681, and MS80. The seven Yamagata strains of 1996 and 1998 were all within the YA2681-related lineage. We reported previously that virus strains which were isolated later than 1981 and had the HE genes of the YA2681 virus lineage can be divided into two distinct subgroups, represented by YA2681 and pig/Beijing/115/81 (PB11581), and that several strains (including Yamagata/10/89 [YA1089], Miyagi/3/91 [MI391], Miyagi/9/91 [MI991], and Miyagi/2/92 [MI292]) having HE genes (as well as other genes) closely related to those of PB11581 were isolated in Yamagata and Sendai cities between 1989 and 1992 (13). It is important to note that the 1996 and 1998 isolates from Yamagata possessed HE genes highly homologous to the genes of these PB11581-like viruses.

To ascertain whether the 1996 and 1998 strains in question have a close relationship with PB11581-like viruses (such as YA1089 and MI991) which circulated in Yamagata and Sendai cities between 1989 and 1992, partial nucleotide sequences of the PB2 (positions 52 to 520), PB1 (positions 50 to 425), P3 (positions 49 to 420), and NP (positions 71 to 670) genes as well as nearly complete sequences of the M (positions 26 to 1,147) and NS (positions 28 to 889) genes were determined for the seven 1996 and 1998 isolates listed above as well as for two old strains (KA176 and MI77), and the phylogenetic trees of individual genes were constructed by using the sequences of these nine strains as well as the previously reported sequences of the 15 strains isolated between 1980 and 1993 (2, 13, 17, 26, 27, 32). For construction of the P3 and NP gene trees, the sequences of the 12 strains isolated in Sendai City between 1993 and 1996 were also included for the reason described below.

As shown in Fig. 3, the PB2 genes analyzed appeared to be split into six lineages, represented by strains MS80, KA176, AI181, SP82, YA2681, and PB11581. The Yamagata isolates of 1996 and 1998, together with YA1089 and MI991, were all located on the PB11581 virus lineage. In the PB1 gene tree, four lineages, represented by AI181, MS80, KA176, and YA2681, were identified, the 1996 and 1998 strains of Yamagata as well as PB11581, YA1089, and MI991 being in the YA2681 virus lineage.

In the tree of the M gene sequences, there were two major branch clusters (previously designated lineages I and II by Tada et al. [32]), one (lineage I) containing KA176, YA2681, and PB11581, and the other (lineage II) containing AI181 and MS80. The 1996 and 1998 isolates in question were within the former branch cluster, which also contained YA1089 and MI991. The NS gene sequences were also split into two discrete lineages (designated A and B by Alamgir et al. [2]), lineage A containing AI181, KA176, and MS80 and lineage B including YA2681 and PB11581. The 1996 and 1998 strains from Yamagata, like YA1089 and MI991, were in the latter lineage. These results indicate that the Yamagata isolates of 1996 and 1998 had PB2, PB1, M, and NS genes (as well HE gene) closely related to those of the PB11581-like viruses that circulated in Yamagata and Sendai cities during 1989 to 1992.

Interestingly, however, the phylogenetic positions of the 1996 and 1998 Yamagata strains were different from those of the PB11581-like viruses in the P3 and NP gene trees. The P3 genes were divided into five different lineages, represented by MS80, KA176, AI181, SP82, and YA2681. The 1996 and 1998 isolates from Yamagata were located on the MS80 virus lineage, separately from PB11581, YA1089, and MI991, which were on the YA2681 virus lineage. The NP genes were split into six distinct lineages, five represented by MS80, YA2681, AI181, KA176, and PB11581 and one (designated the MI193-related lineage) made up by six 1993 and 1994 isolated from Sendai (MI193, MI293, MI693, MI194, MI294, and MI394). The 1996 and 1998 Yamagata strains were located on the MS80 virus lineage, whereas YA1089 and MI991 were on the PB11581 virus lineage. The results shown in Fig. 2 and Fig. 3, taken together, strongly suggest that influenza C virus strains isolated in Yamagata City in 1996 and 1998 are reassortant viruses which acquired HE, PB2, PB1, M, and NS genes from a PB11581-like virus (such as YA1089 and MI991) and P3 and NP genes from an MS80-like virus.

Origin of the P3 and NP genes of influenza C virus strains isolated in Yamagata City in 1996 and 1998. As stated above, the Yamagata isolates of 1996 and 1998 are reassortants which

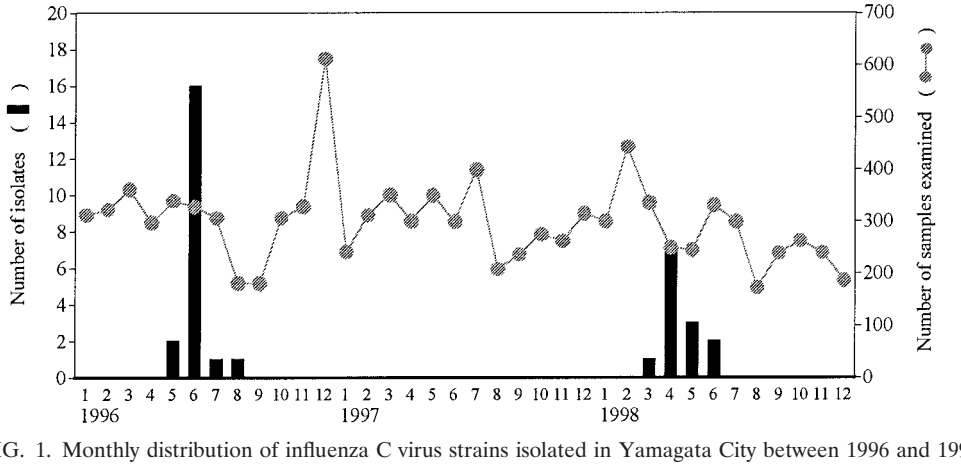


FIG. 1. Monthly distribution of influenza C virus strains isolated in Yamagata City between 1996 and 1998.

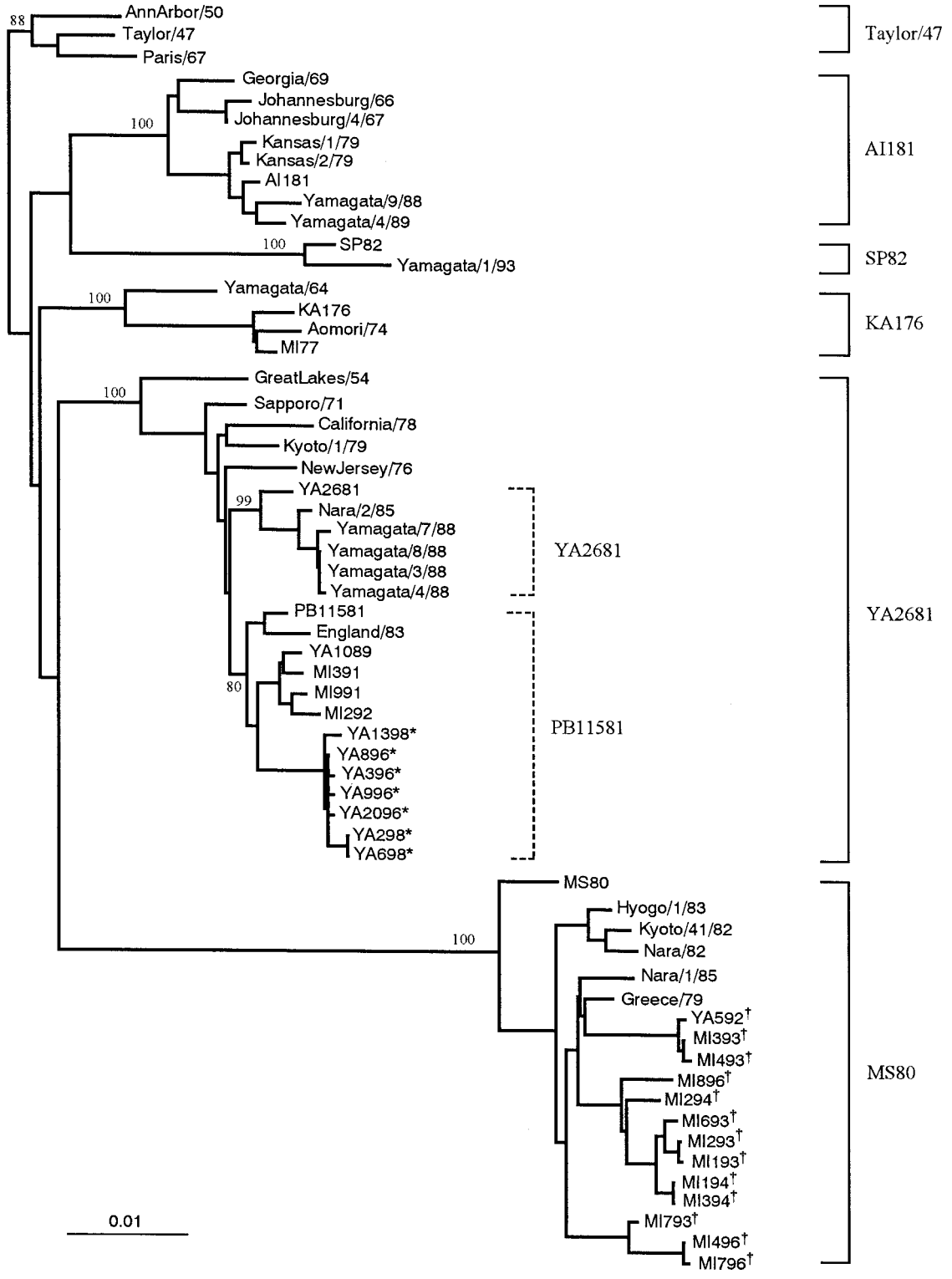
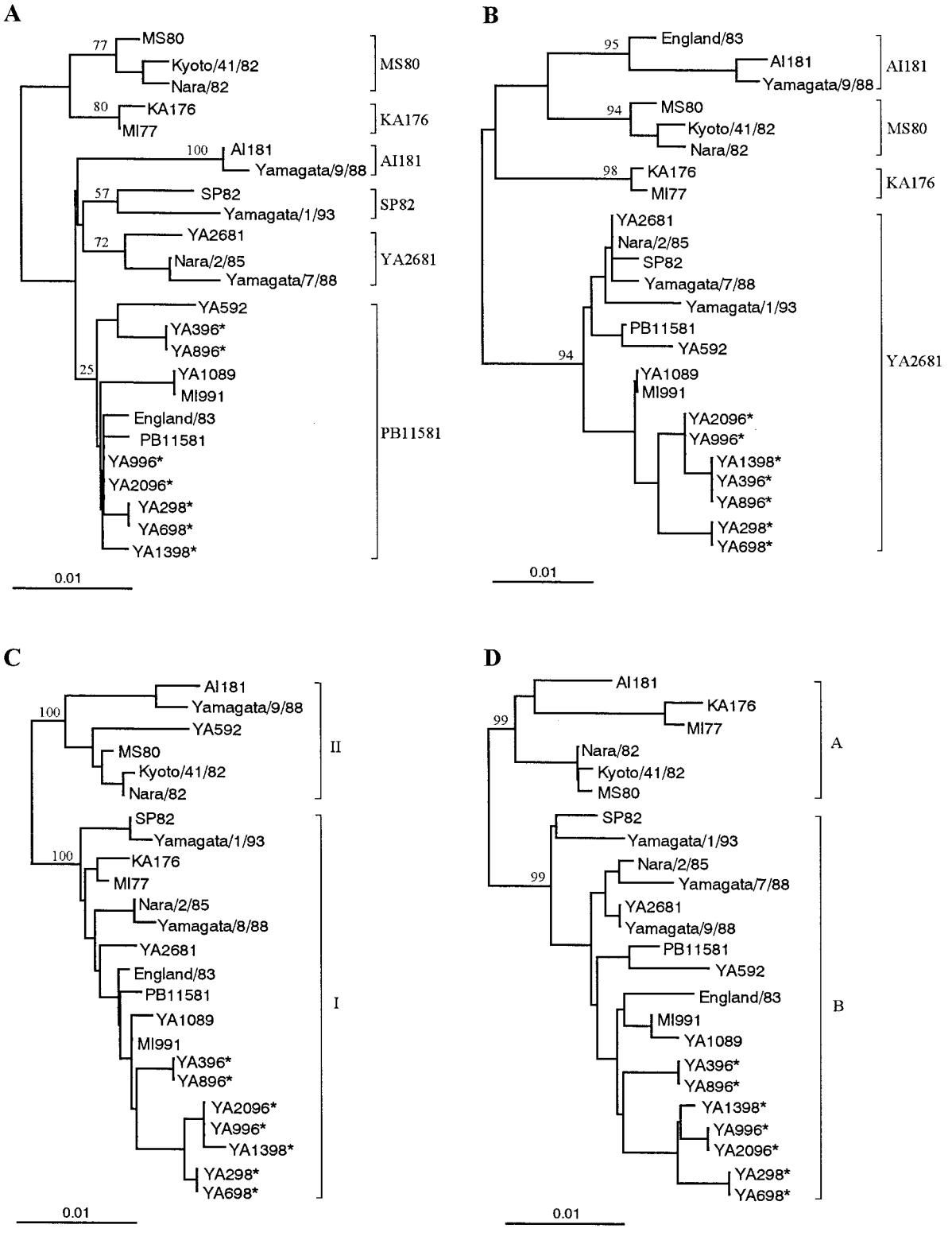


FIG. 2. Phylogenetic tree of influenza C virus HE genes. The region from nucleotides 64 to 1989 was used for analysis. The 1996 and 1998 isolates from Yamagata are marked by asterisks, and Yamagata and Sendai isolates having HE antigenicity identical to that of MS80 are marked by daggers. Horizontal distances are proportional to the minimum number of nucleotide differences needed to join the gene sequences. Numbers below and above the branches are the percent bootstrap probabilities of each branch, determined by the PHYLIP program (version 3.54c).


A
77
MS80
Kyoto/41/82
Nara/82
MS80
80
KA176
MI77
KA176
100
AI181
Yamagata/9/88
AI181
57
SP82
Yamagata/1/93
SP82
72
YA2681
Nara/2/85
Yamagata/7/88
YA2681
YA592
YA396*
YA896*
25
YA1089
MI991
England/83
PB11581
YA996*
YA2096*
YA298*
YA698*
YA1398*
PB11581
0.01
B
95
England/83
AI181
Yamagata/9/88
AI181
94
MS80
Kyoto/41/82
Nara/82
MS80
98
KA176
MI77
KA176
YA2681
Nara/2/85
SP82
Yamagata/7/88
Yamagata/1/93
PB11581
YA592
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YA1089
MI991
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YA396*
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Kyoto/41/82
Nara/82
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Nara/2/85
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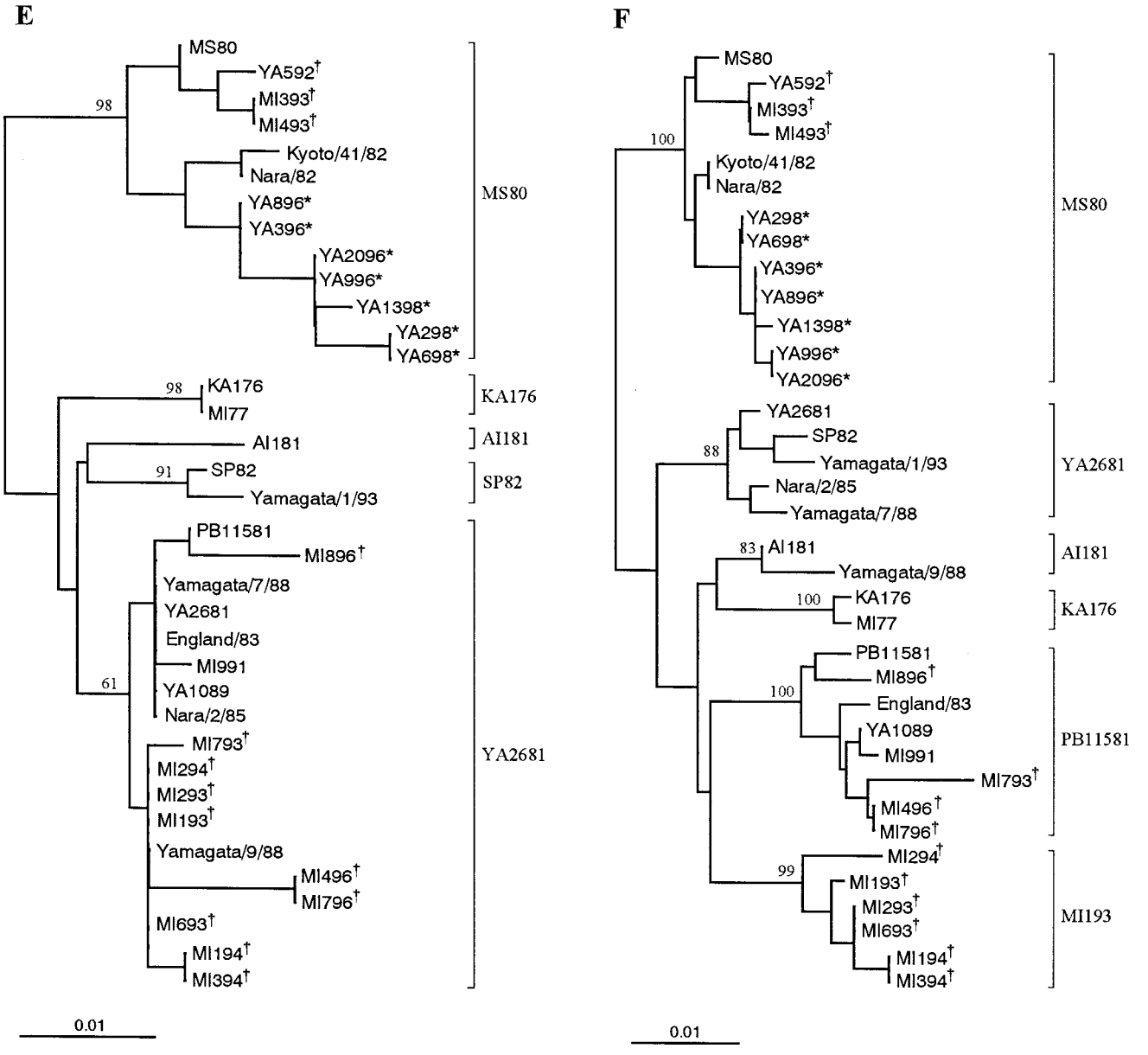

FIG. 3. Phylogenetic trees for the PB2 (A), PB1 (B), M (C), NS (D), P3 (E), and NP (F) genes of influenza C virus isolates. The nucleotide sequences of the following regions were used for analysis: nucleotides 52 to 520 for PB2, nucleotides 50 to 425 for PB1, nucleotides 49 to 420 for P3, nucleotides 71 to 670 for NP, nucleotides 26 to 1,147 for M, and nucleotides 28 to 889 for NS. The 1996 and 1998 isolates from Yamagata are marked by asterisks, and the Yamagata and Sendai isolates having HE genes on the MS80-related lineage are marked by daggers. Horizontal distances are proportional to the minimum number of nucleotide differences needed to join the gene sequences. Numbers above the branches are the percent bootstrap probabilities of each branch, determined by the PHYLIP program (version 3.54c).

obtained P3 and NP genes from an MS80-like parent. In Japan, influenza C viruses having the same HE antigenicity as MS80 were first isolated in the Kinki district between 1982 and 1983 (Kyoto/41/81, Nara/82, and Hyogo/1/83) (1). In the Tohoku district (which includes Yamagata and Miyagi prefectures), however, influenza C virus antigenically indistinguishable from MS80 was isolated for the first time in 1992 (Yamagata/5/92 [YA592]) (27), which was followed by isolation of 12 strains of the MS80 virus group in Sendai City between 1993 and 1996 (six in 1993, three in 1994, and three in 1996; see Materials and Methods).

To obtain information about the origin of the P3 and NP genes of the 1996 and 1998 isolates from Yamagata, the nucleotide sequences of the P3 and NP genes were determined for the 12 MS80-related isolates from Sendai (sequences of YA592 were reported previously [27]) and then compared with those of the Yamagata isolates in question. Figures 2 and 3 show that although all 13 MS80-like isolates (including YA592) had HE genes belonging to the MS80-related lineage, only three (YA592, MI393, and MI493) possessed the P3 and NP genes on the lineages represented by MS80. Moreover, it was found that the older strains (Kyoto/41/82 and Nara/82)

isolated in the Kinki district in 1982, rather than those of the three Yamagata and Sendai strains, had P3 and NP genes more closely related to those of the 1996 and 1998 strains isolated in Yamagata City, raising the possibility that influenza C virus similar to the Kinki strains described above may have supplied the Yamagata strains of 1996 and 1998 with the P3 and NP genes.

DISCUSSION

During a 3-year survey from January 1996 to December 1998, 33 strains of influenza C virus were isolated from 10,726 throat swab specimens collected from children with acute respiratory illness. These 33 strains were isolated in clusters during two different periods, 20 between May and August 1996 and the remaining 13 between March and June 1998. Thus, the isolation rates for June 1996 and April 1998 reached 4.9 and 2.8%, respectively, although the average rate during the 3 years was low (0.3%), as reported by Moriuchi et al. (19). We also found here that all 33 isolates were antigenically and genetically similar and that they were reassortant viruses which arose between two viruses closely related to a PB11581-like virus that circulated in Yamagata and Sendai cities between 1989 and 1992 (13) and an MS80-like virus shown to have circulated in the Kinki district in the early 1980s (1). From these observations, we conclude that two outbreaks of influenza C occurred in Yamagata city in 1996 and 1998, which were each caused by a reassortant virus that had inherited PB2, PB1, HE, M, and NS genes from a PB11581-like virus and P3 and NP genes from an MS80-like virus.

There are several earlier reports which documented influenza C outbreaks that occurred in closed environments such as military induction camps (6, 8), hospitals (7), and children's homes (11, 12, 18). Our surveillance of influenza C virus infections in Yamagata City had succeeded in isolating 27 virus strains by the end of 1995. Although most of them were isolated sporadically, clusters of isolates were observed in December 1988 (five strains) (19). However, antigenic and genetic analyses of these influenza C virus isolates revealed that three of them were closely related to YA2681, whereas the remaining two were similar to AI181 (15; Y. Matsuzaki, unpublished results), which did not allow us to conclude that an influenza C outbreak had been detected. To our knowledge, therefore, this is the first report which describes outbreaks of influenza C detected by long-term surveillance in a relatively large community.

The fact that all 33 strains of influenza C virus isolated in Yamagata City during 1996 to 1998 had antigenic and genetic structures that were virtually identical does not necessarily indicate that influenza C viruses distinct from these isolates were not circulating in Yamagata City during this period. Indeed, in the adjacent city of Sendai, three virus strains with HE antigenicity identical to that of MS80 (MI496, MI796, and MI896) were isolated during the same period, in addition to the 14 strains having the same antigenic and genetic properties as those of the 1996 and 1998 Yamagata isolates (Fig. 2) (Y. Matsuzaki, unpublished results).

In Japan, a PB11581-like virus which provided the 1996 and 1998 strains of Yamagata with five of seven RNA segments was isolated for the first time in Yamagata City in 1989 (YA1089) but was not isolated at all in the city thereafter (13, 15). It seems that a PB11581-like virus acquired the increased ability to prevail in humans by obtaining P3 and NP genes from an MS80-like virus through a reassortment event, resulting in outbreaks of influenza C in Yamagata City in 1996 and 1998. Thus, it seems reasonable to postulate that the genome composition of influenza C viruses influences their ability to spread in humans, and some of the reassortant viruses acquire an epidemiological advantage over their parental viruses and begin to circulate as a predominant strain.

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REFERENCES

1. **Adachi, K., F. Kitame, K. Sugawara, H. Nishimura, and K. Nakamura.** 1989. Antigenic and genetic characterization of three influenza C strains isolated in the Kinki district of Japan in 1982–1983. Virology **172:**125–133.
2. **Alamgir, A. S., Y. Matsuzaki, S. Hongo, E. Tsuchiya, K. Sugawara, Y. Muraki, and K. Nakamura.** 2000. Phylogenetic analysis of influenza C virus nonstructural (NS) protein genes and identification of the NS2 protein. J. Gen. Virol. **81:**1933–1940.
3. **Asahi, Y., K. Sugawara, Y. Muraki, S. Hongo, F. Kitame, and K. Nakamura.** 1997. The antigenic and receptor-binding properties of influenza C viruses adapted to growth in HMV-II cells. Yamagata Med. J. **15:**21–33.
4. **Buonagurio, D. A., S. Nakada, U. Desselberger, M. Krystal, and P. Palese.** 1985. Noncumulative sequence changes in the hemagglutinin genes of influenza C virus isolates. Virology **146:**221–232.
5. **Chakraverty, P.** 1974. The detection and multiplication of influenza C virus in tissue culture. J. Gen. Virol. **25:**421–425.
6. **DeMeio, J. L., R. L. Woolridge, J. E. Whiteside, and J. R. Seal.** 1955. Epidemic influenza B and C in navy recruits, 1953–1954. II. Antigenic studies on influenza virus, type C. Proc. Soc. Exp. Biol. Med. **88:**436–438.
7. **Dykes, A. C., J. D. Cherry, and C. E. Nolan.** 1980. A clinical, epidemiologic, serologic, and virologic study of influenza C virus infection. Arch. Intern. Med. **140:**1295–1298.
8. **Gerber, P., R. L. Woolridge, J. R. Seal, and S. R. Ziegra.** 1952. Epidemic influenza B and C in navy recruits during winter of 1951–52. Proc. Soc. Exp. Biol. Med. **81:**624–628.
9. **Homma, M., S. Ohyama, and S. Katagiri.** 1982. Age distribution of the antibody to type C influenza virus. Microbiol. Immunol. **26:**639–642.
10. **Jennings, R.** 1968. Respiratory viruses in Jamaica: a virologic and serologic study. 3. Hemagglutination-inhibiting antibodies to type B and C influenza viruses in the sera of Jamaicans. Am. J. Epidemiol. **87:**440–446.
11. **Katagiri, S., A. Ohizumi, and M. Homma.** 1983. An outbreak of type C influenza in a children's home. J. Infect. Dis. **148:**51–56.
12. **Katagiri, S., A. Ohizumi, S. Ohyama, and M. Homma.** 1987. Follow-up study of type C influenza outbreak in a children's home. Microbiol. Immunol. **31:**337–343.
13. **Kimura, H., C. Abiko, G. Peng, Y. Muraki, K. Sugawara, S. Hongo, F. Kitame, K. Mizuta, Y. Numazaki, H. Suzuki, and K. Nakamura.** 1997. Interspecies transmission of influenza C virus between humans and pigs. Virus Res. **48:**71–79.
14. **Lamb, R. A., and R. M. Krug.** 2001. Orthomyxoviridae, p. 1487–1531. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, Pa.
15. **Matsuzaki, Y., Y. Muraki, K. Sugawara, S. Hongo, H. Nishimura, F. Kitame, N. Katsushima, Y. Numazaki, and K. Nakamura.** 1994. Cocirculation of two distinct groups of influenza C virus in Yamagata City, Japan. Virology **202:**796–802.
16. **Matsuzaki, Y., M. Matsuzaki, Y. Muraki, K. Sugawara, S. Hongo, F. Kitame, and K. Nakamura.** 1995. Comparison of receptor-binding properties among influenza C virus isolates. Virus Res. **38:**291–296.
17. **Matsuzaki, Y., K. Mizuta, H. Kimura, K. Sugawara, E. Tsuchiya, H. Suzuki, S. Hongo, and K. Nakamura.** 2000. Characterization of antigenically unique influenza C virus strains isolated in Yamagata and Sendai cities, Japan, during 1992–1993. J. Gen. Virol. **81:**1447–1452.
18. **Minuse, E., J. J. Quilligan, and T. Francis, Jr.** 1954. Type C influenza virus. I. Studies of the virus and its distribution. J. Lab. Clin. Med. **43:**31–42.
19. **Moriuchi, H., N. Katsushima, H. Nishimura, K. Nakamura, and Y.**

Numazaki. 1991. Community-acquired influenza C virus infection in children. J. Pediatr. **118:**235–238.
20. **Muraki, Y., S. Hongo, K. Sugawara, F. Kitame, and K. Nakamura.** 1996. Evolution of the hemagglutinin-esterase gene of influenza C virus. J. Gen. Virol. **77:**673–679.
21. **Nakada, S., R. S. Creager, M. Krystal, R. P. Aaronson, and P. Palese.** 1984. Influenza C virus hemagglutinin: comparison with influenza A and B virus hemagglutinins. J. Virol. **50:**118–124.
22. **Nishimura, H., K. Sugawara, F. Kitame, K. Nakamura, and H. Sasaki.** 1987. Prevalence of the antibody to influenza C virus in a northern Luzon Highland village, Philippines. Microbiol. Immunol. **31:**1137–1143.
23. **Nishimura, H., K. Sugawara, F. Kitame, K. Nakamura, N. Katsushima, H. Moriuchi, and Y. Numazaki.** 1989. A human melanoma cell line highly susceptible to influenza C virus. J. Gen. Virol. **70:**1653–1661.
24. **Nishimura, H., S. Hongo, K. Sugawara, Y. Muraki, F. Kitame, H. Washioka, A. Tonosaki, and K. Nakamura.** 1994. The ability of influenza C virus to generate cord-like structures is influenced by the gene coding for M protein. Virology **200:**140–147.
25. **Ohyama S., K. Adachi, K. Sugawara, S. Hongo, H. Nishimura, F. Kitame, and K. Nakamura.** 1992. Antigenic and genetic analyses of eight influenza C strains isolated in various areas of Japan during 1985–9. Epidemiol. Infect. **108:**353–365.
26. **Peng, G., S. Hongo, Y. Muraki, K. Sugawara, H. Nishimura, F. Kitame, and K. Nakamura.** 1995. Genetic reassortment of influenza C viruses in man. J. Gen. Virol. **75:**3619–3622.
27. **Peng, G., S. Hongo, H. Kimura, Y. Muraki, K. Sugawara, F. Kitame, Y. Numazaki, H. Suzuki, and K. Nakamura.** 1996. Frequent occurrence of genetic reassortment between influenza C virus strains in nature. J. Gen. Virol. **77:**1489–1492.
28. **Pfeifer, J. B., and R. W. Compans.** 1984. Structure of the influenza C glycoprotein gene as determined from cloned DNA. Virus Res. **1:**281–296.
29. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. E vol. **4:**406–425.
30. **Sugawara, K., F. Kitame, H. Nishimura, and K. Nakamura.** 1988. Operational and topological analyses of antigenic sites on influenza C virus glycoprotein and their dependence on glycosylation. J. Gen. Virol. **69:**537–547.
31. **Sugawara, K., H. Nishimura, S. Hongo, Y. Muraki, F. Kitame, and K. Nakamura.** 1993. Construction of an antigenic map of the haemagglutinin-esterase protein of influenza C virus. J. Gen. Virol. **74:**1661–1666.
32. **Tada, Y., S. Hongo, Y. Muraki, K. Sugawara, F. Kitame, and K. Nakamura.** 1997. Evolutionary analysis of influenza C virus M genes. Virus Genes **15:**53–59.
33. **Umetsu, Y., K. Sugawara, H. Nishimura, S. Hongo, M. Matsuzaki, F. Kitame, and K. Nakamura.** 1992. Selection of antigenically distinct variants of influenza C viruses by the host cell. Virology **189:**740–744.